Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

Properties of Immobilized Pectinesterase on Nylon

Selma Ates[a]; Sule Pekyardimci[a]
[a] Department of Chemistry, Faculty of Sciences, Ankara University, Ankara, Turkey

To cite this Article Ates, Selma and Pekyardimci, Sule(1995) 'Properties of Immobilized Pectinesterase on Nylon', Journal of Macromolecular Science, Part A, 32: 1, 337 — 345
To link to this Article: DOI: 10.1080/10601329508019179
URL: http://dx.doi.org/10.1080/10601329508019179

MACROMOLECULAR REPORTS, A32(SUPPL. 3), 337-345 (1995)

PROPERTIES OF IMMOBILIZED PECTINESTERASE ON NYLON

Selma Ateş, Şule Pekyardimci
Department of Chemistry, Faculty of Sciences, Ankara University,
06100 Ankara Turkey

ABSTRACT

Pectinesterase (PE) was immobilized by covalent attachment on nylon-polyethylenimine co-polymer and the properties of the immobilized enzyme was investigated. The nylon support was activated both by using dimethyl sulphate (DMS) and triethyloxonium tetrafluoroborate (TOTFB). The suitable conditions for an operative and stable system were investigated. The immobilization of PE on nylon which had been by polyethylenimine (PEI) resulted in some loss of activity because of steric hindrance of pectin. The activity immobilization yielded 8.2 % for DMS and 12.7% for TOTFB and higher than the previously reported in the literature. The pH optima and temperature stability of the immobilized PE significantly increased.

INRODUCTION

Immobilization of various enzymes has been studied in detail during the past 35 years. The advantages of using immobilized enzymes in food, drug and analytical applications are well known [1,2]. There are some disadvantages of immobilization techniques which need to be minimized prior to large scale applications. A decrease in enzymatic activity is observed in all entrapting methods, due to the hindrance caused on diffusion of substrate by the polymer matrix [3].

Pectin is a generic name for high molecular weight polysaccharides present in higher plants that are responsible of the

consistency and turbidity. Pectin degradation by using pectic enzymes constitutes a common industrial practice to achieve clarification [4,5,6]. The main pectic enzymes, pectinesterase (PE) and polygalacturonase (PG) may be used either individually or sequentially to clarify [7]. Several attempts have been carried out in order to obtain highly-active and stable insoluble derivatives of these enzymes [8]. Pectinesterase seemed to be more sensitive to the effects of immobilization than polygalacturonase [9]. The highest relative activity of covalently bounded PE on CNBr-activated Sepharose 4B was only 7.3 % [10].

Macromolecular supports can offer considerable advantages in industrial applications of immobilized enzymes. The use of non-porous support with improved mechanical and hydrodynamic characteristics, as well as possibilities of chemical modification, by using nylon, would be advantageous for the hydrolysis of a high molecular weight substrate with a colloidal character, furthermore nylon is relatively low cost. The chemical processes used to activate nylon involves cleavage of the polyamide chain for the release of free amino groups to which the enzymes were covalently bound through glutaraldehyde [11].

In this paper we report on the preparation of an immobilized PE using nylon-66 pellets as support. Nylon has been coated with polyethylenimine in order to obtain a large number of potentially active amino groups for the subsequent binding of enzyme molecules [12] The nylon support was actived by using both dimethyl sulphate (DMS) and triethyoxonium tetrafluoroborate (TOTFB). The kinetic properties and stability of the various preparations are reported.

MATERIALS AND METHODS

Materials : Pectinesterase (EC 3.1.1.11) from tomato, citrus pectin (methoxy content 8.6 %), polyethylenimine, methylen chloride were all purchased from Sigma Chemical Company (USA). All the remaining reagents were obtained from Aldrich Chemical Company (USA). All

reagents were analytical grade and were used without further purification. Nylon-66 pellets (3mm particle size) were supplied by Tofaş (Turkey)

Immobilization Process : Pectinesterase was immobilized on nylon-66 pellets by an o-alkylation activation methods developed and previously described in detail by Thompson et al. In brief the process was as fallows: Support activation with DMS and TOTFB. Then the activated support was coated with PEI. The nylon-PEI derivative was reactivated with glutaraldehyde to which the pectinestease was finally attached.

1.25 % (W/V) glutaraldehyde solution, freshly made up in 0.1 M $NaHCO_3$ buffer, at pH 9.4. Buffer solutions were prepared from common solid reagent grade chemicals and distilled water.

Pectinesterase Activity Measurement: The activity of PE was determined by a modification of the method Rouse and Atkins (1955) [9]. Titration method of enzymatically liberated carboxyl groups from pectin using 0.01 M NaOH in a titration set with pH metre (Pye-Unicam). The substrate was 1 % citrus pectin in 0.1 M NaCl and the assay was carried out at pH 7.0 at temperature 30 °C. Continuous stirring was applied to homogenize the mixture.

The enzyme activity of PE as a function of pH was examined over the pH range 4-10 in acetate and phosphate buffers. The temperature stability was determined from the activity values (at the pH optimum) after 20 minutes incubation of the enzymes at various temperature (30-70 °C).

One Unit of enzyme activity was defined as the amount of enzyme able to generate one micromole of H^+ per minute at the standart conditions.

RESULTS AND DISCUSSION

In all the experiments performed hitherto about PE immobilization was used only DMS to activate nylon supports. In this research has been used TOTFB instead of DMS and the immobilization yields were higher than DMS activated nylon. Effect of activation nylon by treatment with DMS and TOTFB on PE has been shown in Table 1.

For several reasons TOTFB is a preferable reagent for this operation as it is more efficient and does not have the disadvantages associated with DMS. First, alkylation of nylon with the latter reagent was carried out with undiluted reagent for 4 minutes at 100 °C. On the otherhand dilute solutions of TOTFB in

TABLE 1: Comparison of Activation of nylon by treatment with DMS and TOTFB

	Removed Activity Ratio(%)	Coupling Efficiency(%)	Activity immobilization yield(%)	Derivative Activity(U g^{-1})
DMS	58.1	14.1	8.2	11.4
TOTFB	68.6	18.6	12.7	17.7

Removed Activity Ratio: Ratio of the activity removed from the solution during the immobilization process to the total units of soluble enzyme used for immobilization.
Coupling Efficiency : Ratio of enzymic activity shown by the immobilized enzyme to the activity removed from the solution during the immobilization process.
Activity Immobilization Yield: Ratio of enzymic activity shown by the immobilized enzyme to the total units of soluble enzyme.
Activity of the Derivative: Units of enzymic activity shown per gram of derivative.

dichloromethane alkylates nylon smoothly at room temperature, and the extent of alkylation can be controlled to give a more reproducible product. Secondly, although TOTFB must be treated as poisonous it is a safer reagent than DMS since it does not give off poisonous vapours and does not cause burns.

As can be seen in Table 1 all assayed activities showed low efficiency and activity immobilization yields. But these values were in of higher than those obtained for different immobilized pectolitic enzyme derivatives developed by other authors. This has been attributed to the high dependence of activity immobilization yields of pectolitic enzymes derivatives on the molecular weight of substrate; the severe sterical restrictions imposed by the matrix limits the cleavage of the glycosidic bonds of the substrate to the more external accessible linkages, this implies that the cleavage of

the substrate by immobilized PE follows a more "exo" mechanism than that of the corresponding soluble enzymes[14]

pH activity profile : The effect of pH on free and immobilized PE activity was examined over the pH range 4-10 in acetate and phosphate buffers. Figure 1. shows the pH activity profiles for the activity of PE immobilized on nylon-PEI copolymer as compared with those of the soluble enzyme preparation. The maximum activity of the immobilized PE was obtained at pH 7, being coincident with that for the soluble enzyme. Between pH 4-7 immobilized PE activity was higher than soluble enzyme. The acidic pH value obtained is interesting industrially because the majority of the natural juices show a pH between 3.5-5.5[15]. This means that the enzyme preparation will work under pH 7, thus increasing the operational stability. Additionally, a low operational pH makes microbial contamination of both the substrate products and the enzyme reactor more difficult.

Effect of Temperature on Activity: The temperature stability was determined from the activity values (at the pH 7) after 20 minutes incubation of the enzymes various temperature. The results are shown in Figure 2. All preparations of immobilized PE showed increased temperature optima and increased temperature stability when compared with the free enzymes.

Storage Stability: Both soluble and immobilized PE activity in phosphate buffer at pH 7.0 were fallowed during storage at 4 °C for 30 days. The immobilized PE activated with TOTFB and DMS retained 100 % activity after 30 days. But as seen in Figure 3 there is a derease in soluble enzyme activity.

Reuse Number: Maximum activity versus reuse number plots of immobilized enzyme on nylon activated both with TOTFB and DMS are shown in figure 4. As seen in the plots there is a decrease in both immobilized enzymes activity after first use which does a slight change afterwards. The stability in activity even after 13th use

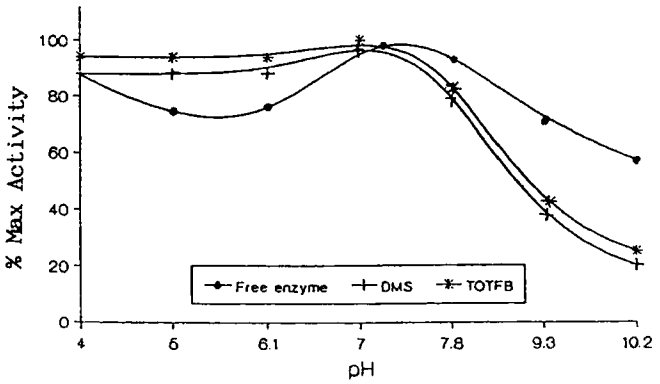

Figure 1. Effect of pH

$$\% \text{ Max. Activity} = \frac{\text{Coupling efficiency} \times 100}{\text{M.a}}$$

M.a : Maximum value of coupling efficiency.

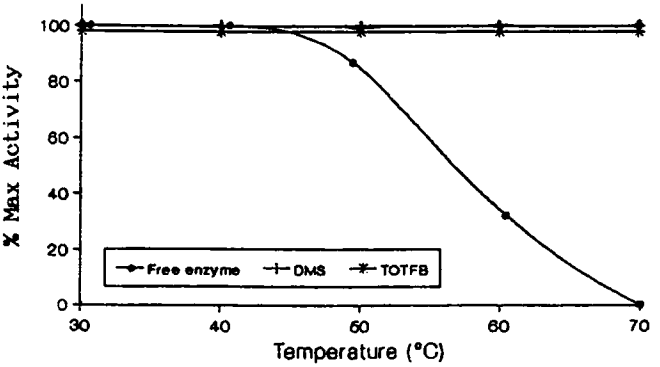

Figure 2. Effect of temperature

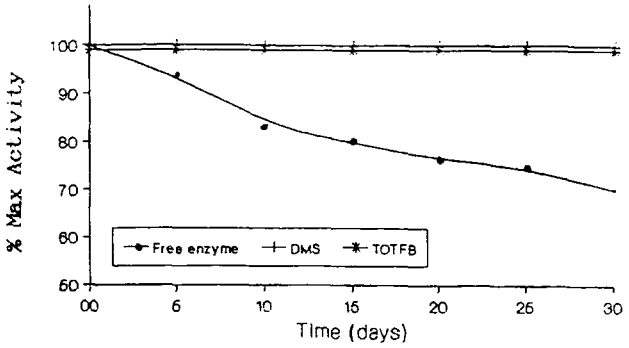

Figure 3. Storage stability of immobilized and soluble PE

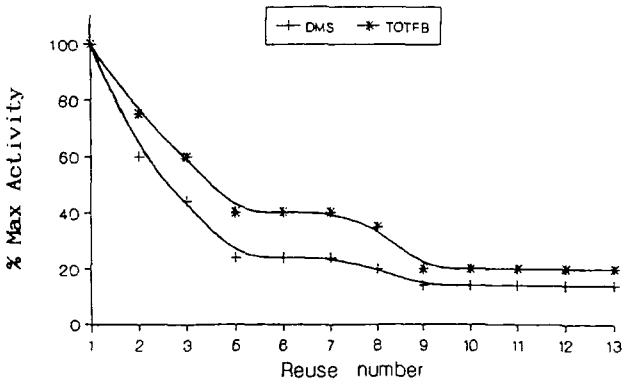

Figure 4. The effect of reuse number of immobilized enzyme on nylon activated with TOTFB and DMS

TABLE 2: Kinetic parameters of pectinesterase

Enzyme Form	Vmax (U/mg)	Km (mg/ml)
Immobilized on nylon activating with DMS	29.4	20.0
Immobilized on nylon activating with TOTFB	36.7	13.3
Soluble enzyme	142.2	4.0

shows that pectinesterase is immobilized mainly by covalent bonding rather than physical entrapment.

Kinetic Constants: The kinetic constants of free and immobilized PE were calculated from the initial rates at various substrate concentrations. The results are shown in Table 2. The enzyme, both soluble and immobilized fallowed a Michaelis-Menten behaviour when using citrus pectin as substrate. The enzyme immobilization resulted in an increase of the Michaelis constant for citrus pectin. This increase is usually related to high steric limitations caused by the support structure.

REFERENCES

1. Axen R., Salmona M., Soronio C. and Garattini S. Methods for Preparing İmmobized Enzymes New York: Raven Pres (1974)

2. Zaborsky O. Immobilized Enzymes Ohio: CRC Press (1973)

3. Kwinkova M. and Kucera J. Enzyme Microb. Technol. 5, 204-208 (1983)

4. Weibel M.K., Borrios R., Delloto R. and Humprey A.E. Biotechnol. Bioeng. 17, 85-98 (1975)

5. Vijayalakshmi M.A., Picque D., Janmoville R., Sagard E. Food Process Eng. 2, 152-154 (1980)

6. Lozano P., Manjon A., Romojaro F., Canovas M. and Iborra J.L. Biothechnology Letters 9, 12, 875-880 (1987)

7 Borrego F., Tari M., Majon A. and Iborra J.L. Applied Biochemistry and Biotechnology 22, 129-140 (1989)

8 Lozano P., Manjon A., Iborra J.L., Canovas M., Romojaro F. Enzyme Microb. Technol. 12, 499-505 (1990)

9. Lozano P., Manjon A., Iborra J.L. and Romojaro F. Process Biochemistry 75-78 (1988).

10. Markovic O., Machova E. Coll. Czech Chem Commun. 50, 2021-2027 (1985)

11. Morris D.L., Campbell J. and Hornby W.E. Biochem. J. 147, 593-603 (1974)

12. Thompson R.O., Mandoke C.S. and Womack J.P. Anal. Letters 18, 93-107 (1985)

13. Rouse A.H. and Atkins C.D. Agric Exp. Stn. Bulletin 570 (1955)

14. Rexova B.L. and Merakova D.M. Carbohyd. Res. 98, 115 (1981)

15. Klavons J.A. and Bennet R.D. J. Agric. Food Chem. 35, 1 (1987)